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ORIGINAL RESEARCH

Microbial Carriage of Cockroaches at a Tertiary Care Hospital in Ghana

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Abstract: Cockroaches are common in the environment of many hospitals in Ghana; however, little is known about their public health risks. To evaluate potential risks, we investigated the external and internal microbial flora of 61 cockroaches from a tertiary hospital in Ghana and evaluated the antibiotic resistance profiles of the common bacterial species. Standard methods were used in all the microbiological investigations and antibiotic susceptibility testing. A rotavirus carriage rate of 19.7% was observed among the cockroaches. Four types of intestinal parasites were carried externally by the cockroaches, and the most prevalent was Hookworm (4.9%). Eight nosocomial bacteria were isolated from the cockroaches, and the most prevalent was *Klebsiella pneumoniae*, which occurred internally in 29.5% of the cockroaches and 26.2% externally. Multiple drug resistance among common bacteria isolated from the cockroaches ranged from 13.8% (*Escherichia coli*) to 41.1% (*Klebsiella pneumoniae*). Cockroaches constitute an important reservoir for pathogenic microorganisms, and may be important vectors of multiple resistant nosocomial pathogens in the studied hospital.

Keywords: cockroach, *Klebsiella pneumoniae*, hookworm, rotavirus, antibiotic resistance, nosocomial

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Introduction

Cockroaches are of public health significance as they can serve as hosts, reservoirs and carriers of pathogenic microbes.[1–3] Cockroaches feed readily on feces, sputum, skin scrapings and other human detritus as well as on a variety of foodstuffs. The filthy behavior of cockroaches coupled with their nocturnal lifestyle make them ideal vectors for a wide range of pathogens such as *Escherichia coli, Pseudomnonas aeruginosa, Klebsiella* spp., *Shigella dysenteriae*, *Bacillus cereus*, and *Entamoeba histolytica*.[1–4] Usually cockroaches dwell in crevices of buildings and garbage, and improper handling of refuse as well as urbanization have been linked with increased infestation of cockroaches in communities.[5,6] In developing countries, overcrowding and poor sanitary conditions, which are a common characteristic in most public health care settings, provide favorable breeding grounds for cockroaches.[3,4,6–8]

Cockroaches pose a concern in the hospital environment because they may serve as reservoirs for nosocomial pathogens.[1,3,5–9] Nosocomial infections constitute a major public health burden with serious economic consequences, which are exacerbated by antimicrobial resistance among the etiological microorganisms.[10,11] In developing countries such as Ghana, there have been concerns about the increasing cockroach populations in some hospitals.[4,7,9,12] However, no study has investigated the public health risks posed by cockroaches in Ghanaian hospitals. The only study that investigated microbial carriage of cockroaches in Ghana was carried out in kitchens and villages, and identified mainly *Staphylococcus aureus* and enterobacteria.[13] As this study investigated only diarrheal bacterial microorganisms, the spectrum of microbial agents carried by cockroaches in Ghana, especially in the hospital environment, still remain unknown. In the current study, we found that cockroaches at the Korle-Bu Teaching Hospital, a major referral center in Ghana, constitute an important reservoir of rotavirus and a range of parasites and bacterial species, many of which are of nosocomial significance. Our findings indicate that cockroaches at the hospital constitute an important source of multiply resistant bacteria.

Materials and Methods

Sampling, identification and processing of cockroaches

The study was carried out at the Korle-Bu Teaching Hospital in May, 2012. Cockroaches at the Korle-Bu Teaching Hospital were collected using sticky rods, traps, and using a direct collection method with sterile hand-gloves.[14] The cockroaches were collected once a week for 4 weeks. The sampled cockroaches were transported to the Microbiology laboratory of the University of Ghana Medical School and identified using the method described by Burgess in 1993.[15] Live, intact and matured cockroaches were processed for microbiological investigations as follows. The cockroaches were killed in a sterile jar using cotton soaked in chloroform. Each cockroach was then placed in a beaker and washed in 5 mL sterile physiological saline by shaking for 2 minutes. The wash was considered as external body homogenate sample. Following the external body washing, the cockroach was soaked in 90% ethanol for 5 minutes to decontaminate the body surface and taken out of the ethanol to dry. It was then re-washed with sterile physiological saline to remove traces of ethanol, and the alimentary tract was aseptically dissected out using autoclave-sterilized entomological dissecting needles. The excised gut was then homogenized in 5 mL of sterile saline solution, and the sample was considered an internal body homogenate sample.

Parasitological examination

About 2 mL of both the external and internal homogenate of the saline washes were dispensed into centrifuge tubes and centrifuged with a g-force of 1258 g at 5000 rpm for at least 5 minutes. The sediments were examined using the direct wet mount.[16] Briefly, a drop of the suspended sediment was placed on 2 clean, grease-free microscope slides, and stained with 1% Lugol's iodine. Each slide was then examined for parasites under the light microscope.

Virological examination

Homogenates from all the samples were tested for rotavirus using the ProSpecT TM Rotavirus ELISA kit (Oxoid, UK). The process was carried out using the protocol designed by the manufacturer (Oxoid, UK). In order to ensure that quality and accurate results were obtained, known positive rotavirus sampling (positive control test) and sterile water tests (negative control test) were also run on the rotavirus kits.

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Isolation, identification and antibiotic susceptibility testing of bacteria

The external and gut (internal) homogenates were enriched in buffered peptone water and incubated overnight at 37 °C. The enriched samples were subcultured onto various primary media including Mac-Conkey agar, Sheep Blood Agar, Chocolate agar and Deoxycholate Citrate agar. The agar plates were incubated overnight, and isolated colonies were identified based on colonial morphology, Gram staining and a battery of biochemical reactions, such as the triple sugar iron test, catalase test, urease test, indole test and citrate utilization test.[17–21]

Identified bacterial colonies were purified and their susceptibility patterns were determined for various antibiotics using a modified form of the Kirby Bauer method.[18,19] The antibiotics tested included gentamicin, amikacin, tetracycline, cotrimoxazole, cefotaxime, ceftizoxime, ampicillin, piperacillin, chloramphenicol, ciprofloxacin, levofloxacin and ofloxacin (Oxoid Ltd., Basingstoke, UK). These antibiotics were tested because they seem to be common on the Ghanaian market. The antibiotic susceptibility testing procedure employed is briefly described as follows. The test organism was emulsified in peptone water until the turbidity was comparable with 0.5% McFarland's standard. A loopful of the suspension was transferred onto a Mueller-Hinton agar plate, and then a sterile cotton swab was used to streak the entire surface of the plate. Sterile forceps were used to apply the antibiotic discs to the surface of the agar plate and incubated at 37 °C for 18–24 hours (h). Zone diameters around the antibiotic discs were measured and classified as sensitive or resistant based on the NCLS break point system.

Statistical analysis

The data collected in the study were entered into MS Excel and analyzed using Minitab software version 15 (Minitab Inc., 2010). Descriptive analysis was carried out on the various microbial agents carried by cockroach samples, including determination of their frequencies of occurrence and prevalence rates. Subsequently, a Chi-square test was used to compare external and internal carriage rates of the different microbial agents at $P < 0.05$. Antibiotic resistance data of each bacterial species was categorized as "resistant isolates" and "sensitive isolates". Subsequently, for each bacterial agent, the frequency and prevalence of resistant isolates were determined. Isolates that were resistant to 3 or more antibiotics were labeled as multidrug resistant isolates; the frequency and prevalence of such isolates were also determined.

Results

All 61 cockroaches sampled in the study were identified to be *Periplaneta americana*. 12 (19.7%) of the cockroaches tested positive for rotavirus. Parasitological investigations identified 4 different parasites carried by 6 (7%) cockroaches (Table 1). All the parasites occurred on the external surface of the cockroaches and the most common was *Ancylostoma duodenale* (4.9%). Bacteria were isolated from all 61 cockroaches. Overall, 8 bacterial species were isolated and each bacteria was isolated from both the internal and external parts of the cockroaches (Table 1). Prevalence rates of the bacteria from the 2 cockroach parts were 1.3%–26.2% and 1.3%–29.5%, respectively, and there was no significant difference in bacteria isolated from the 2 cockroach parts. The most common bacteria isolated was *Klebsiella pneumoniae,* followed by *Escherichia coli*, *Proteus vulgaris*, *Citrobacter ferundii*, *Enterobacter cloacae*. Some bacteria agents, notably *Enterococcus faecalis* and *Pseudomonas aeruginosa,* were relatively less commonly carried by the cockroaches (<5%).

Table 1. Bacteria and parasites carried externally and internally by cockroaches.

Microorganism	External	Internal
	n (%)	n (%)
Bacteria		
Klebsiella pneumoniae	16 (26.2)	18 (29.5)
Escherichia coli	15 (24.6)	14 (23.0)
Proteus vulgaris	14 (23.0)	13 (21.3)
Citrobacter ferundii	13 (21.3)	14 (23.0)
Enterobacter cloacae	8 (13.1)	10 (16.4)
Pseudomonas aeruginosa	3 (3.9)	2 (3.3)
Enteterococcus faecalis	1 (1.3)	2 (3.3)
Klebsiella oxytoca	1 (1.3)	1 (1.3)
Parasites		
Ancylostoma duodenale larvae	3 (4.9)	0 (0)
Intestinal flagellate	1 (1.6)	0 (0)
Taenia spp.	1 (1.6)	0 (0)
Hymenolepis nana	1 (1.6)	0 (0)
Virus		
Rotavirus	12 (19.7)	12 (19.7)

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Antibiotic resistance patterns of the common bacteria isolated from the cockroaches are reported in Figure 1. For *Klebsiella pneumoniae*, the percentage resistance ranged from 6% (piperacillin) to 88% (cotrimoxazole). Among isolates of *Citrobacter freundii*, the percentage resistance ranged from 8% (levofloxacin/piperacillin) to 61.5% (tetracycline); no resistance was observed for gentamicin. *Enterobacter cloacae* also showed percentage resistance ranging from 37.5% (chloramphenicol/ceftizoxime/gentamicin) to

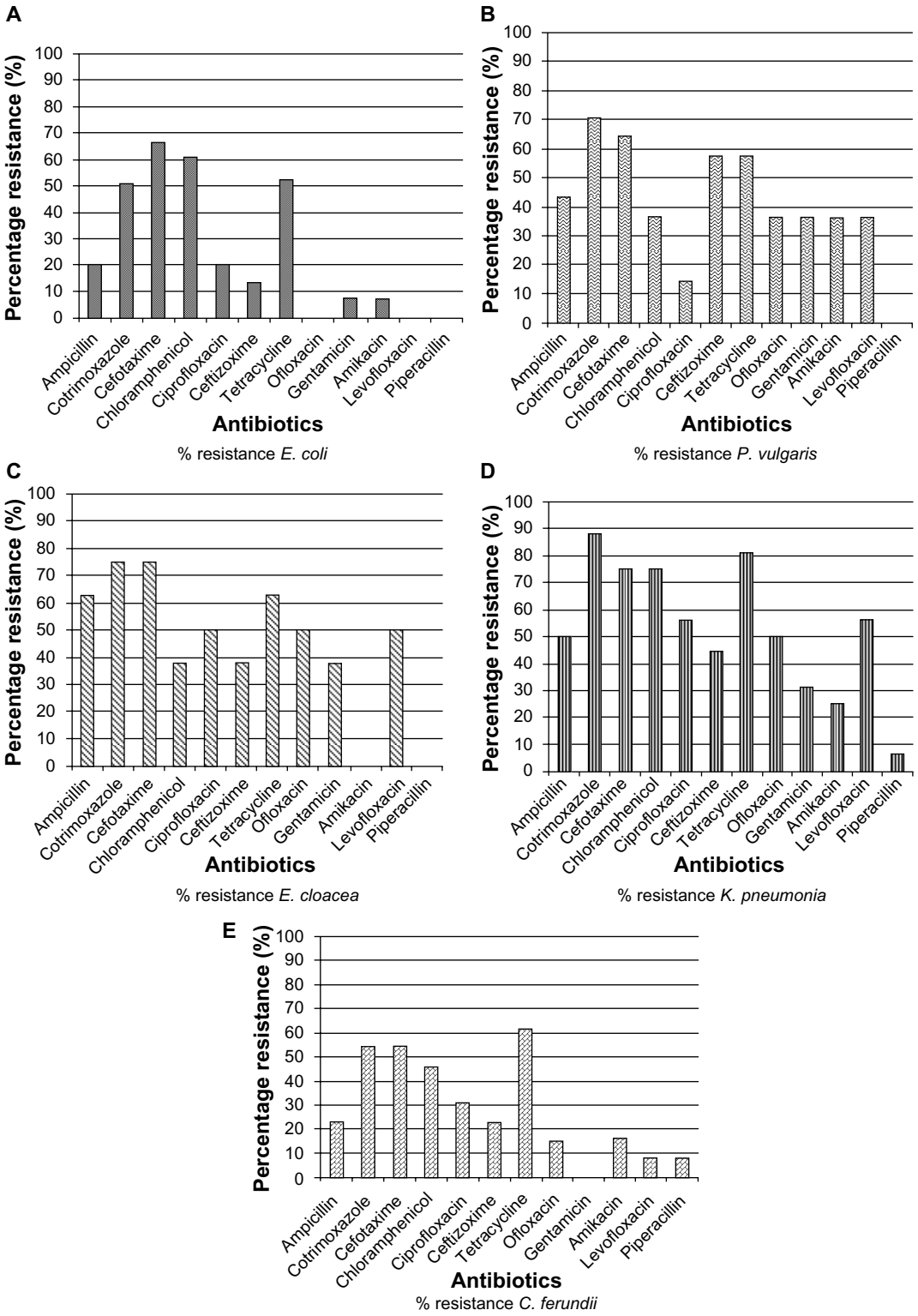

Figure 1. Antibiotic resistance prevalence rates of common bacterial species isolated from cockroaches.

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75% (cotrimoxazole/cefotaxime); no resistance was observed for amikacin and piperacillin. The percentage resistance for *Escherichia coli* ranged from 6.7% (amikacin) to 66% (cefotaxime); no resistance was observed for levofloxacin, piperacillin and ofloxacin. Isolates of *Proteus vulgaris* also displayed percentage resistance ranging from 14% (ciprofloxacin) to 70% (cotrimoxazole); no resistance was observed for piperacillin. The prevalence of multiple drug resistance among the common bacterial species isolated were *Klebsiella pneumoniae* (41.1%), *Citrobacter ferundii* (29.6%), *Proteus vulgaris* (29.6%), *Enterobacter cloacae* (27.8%) and *Escherichia coli* (13.8%).

Discussion

In this study we investigated microbial flora carried by cockroaches collected from a tertiary care hospital in Ghana. A wide range of bacterial species were isolated from the cockroaches sampled, an observation which has also been reported in other studies (Table 2). This highlights the close association of cockroaches and bacteria, which is not surprising given the broad habitat range of cockroaches and the ubiquitous nature of bacteria. Interestingly, the bacterial flora carried by the cockroaches in this study is similar to that reported by a study in the UK.[5] In both studies, cockroaches carried *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterobacter specie, Proteus* specie *and Klebsiella* specie. Most of these organisms have also been isolated from cockroaches in France and India,[8,9] suggesting that the type of bacterial flora carried by cockroaches may be independent of geographical location or socio-economic factors. Most of the bacteria carried by cockroaches in this study are pathogenic or potentially pathogenic, and are therefore of public health significance. For example, the most prevalent bacterial agent, *Klebsiella pneumoniae,* causes pneumonia, urinary tract infections and wound infections.[20,21] *Proteus vulgaris*, an opportunistic pathogen, causes urinary tract and wound infections.[20,21] Similarly, *Citrobacter freundii*, another opportunistic pathogen, causes respiratory infections, urinary tract infections and septicemia.[20,21] The overall bacterial flora of the cockroaches sampled is highly related to nosocomial infections, as all 8 bacteria identified are recognized nosocomial pathogens. In a previous study carried out at the Korle-Bu Teaching Hospital, various fomites and hospital equipment were found to be reservoirs of nosocomial pathogens.[22] The current study, which was carried out in the same hospital, shows that apart from fomites and hospital equipment, infestation of the hospital environment with cockroaches constitutes an important reservoir of nosocomial pathogens. In addition to being reservoirs of nosocomial pathogens in the hospital, the cockroaches can also spread the nosocomial pathogens in the hospital environment,

Table 2. Microbial carriage of cockroaches reported by other investigators.

Other works	n	Site	Organisms isolated
Fotedar et al[1]	96	Hospital ward	*Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis* and *Micrococci*
Tachbele et al[4]	600	Four hospitals and four restaurants	*Salmonella* spp., *Shigella flexneri, Escherichia coli O157, Staphylococcus aureus* and *Bacillus cereus*
Oothuman et al[7]	104	Four paediatric wards	*Shigella boydii, S. dysenteriae*, *Salmonella typhimurium, Klebseilla oxytoca, K. ozaena* and *Serratia marcescens*
Guyader et al[8]	532	Hospital environment	*Citrobacter freundii, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumonia, Enterobacter agglomerans, Escherichia adecarboxylata, Serratia marcescens, Serratia liquefaciens. Acinetobacter, Pseudomonas fluorescens, P. putida,* and *P. aeruginosa. Escherichia coli, Staphylococcus aureus*
Fotedar et al[9]	279	Hospital environment and residential area	Bacteria: *Klebsiella, E. coli, Enterobacter, P. aeruginosa, proteus* spp., *S. aureus; S. epidermidis, S. faecalis, S. viridans; micrococci, streptococci* and *Bacillus.* Parasites: *Endolimax nana, Entamoeba coli, Entamoeba histolytica* Fungi: *Candida* spp., *Rhizopus* spp. *Mucor* spp., *Alternaria* spp. *Aspergillus niger, Aspergillus flavus, Aspergillus* spp.

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including transferring the organisms from one fomite to another. This probably makes infected cockroaches relatively more important reservoirs of nosocomial pathogens, and their presence in healthcare environments should be seen as a serious public health threat. Though we did not collect fecal specimens of cockroaches in this study, the common occurrence of the 8 bacterial microorganisms in the gut of the cockroaches indicate that their feces could also pose a public health risk.

For the various bacteria isolated from the cockroaches, a high percentage of resistance was observed for tetracycline, cotrimoxazole and cefotaxime. Generally, this concurs with resistance data of the various bacteria isolated from patients and healthy individuals in Ghana.[23–25] These antibiotics, especially tetracycline and cotrimoxazole, have been on the Ghanaian market for a long time and therefore have been subjected to high rates of antibiotic use or abuse, hence their high levels of resistance observed.[23,24] The high prevalence of cefotaxime resistance in this study (54%–75%) is surprising, as a previous study at Korle-Bu Teaching Hospital reported an overall prevalence of 18%.[23] This study involved most of the bacteria in the current study, but this was isolated from patients. Over a 10-year period, resistance to this antibiotic seems to have increased by 36%–57%. This highlights the rapid rate of evolution of antibiotic resistance in healthcare settings where there are no strict regulations regarding antibiotic prescription and use. The result is a high prevalence of multiple drug resistance (13.8%–41.1%) observed among the common bacterial pathogens, which threaten antimicrobial therapy and could have serious implications in a developing country like Ghana, where antibiotic treatment options are relatively limited.

To the best of our knowledge, this is the first study to report on viral carriage of cockroaches, though we investigated only one viral agent due to our limited resources. We found 19.7% carriage of rotavirus, which is quite high, considering the public health importance of this viral agent in Ghana. Rotavirus is the most common cause of severe and fatal diarrhea in young children worldwide, and in Ghana almost half of the diarrheal disease hospitalizations of children under 5 are caused by rotavirus.[26,27] The common occurrence of cockroaches in Ghanaian homes and their association with food suggests that cockroaches could play a role in transmission of rotavirus infection. However, this possibility requires further investigation.

As far as parasites are concerned, the cockroaches sampled appear to be important mainly for gastrointestinal infections, as all 4 parasites identified are gastrointestinal parasites.[28] The most prevalent parasite among the cockroaches, *Ancylostoma duodenale* (hookworm), has been commonly reported in some economically disadvantaged communities in Ghana.[29,30] Hookworm infection is transmitted by skin penetration. Therefore, the presence of the larval stages on these cockroaches can pose health problems, as the cockroaches can deposit the larvae at various places, and on items that can get into contact with human skin. Since the egg of *Taenia solium* could lead to human cysticercosis, an extra-intestinal complication of *Taenia* spp. infection, the current finding will help create awareness, which would eventually lead to avoiding this complication. A report by the Center for Food Security and Public Health (USA) indicated that neurocysticercosis is the most serious form of cysticercosis, which occur when the there is a blockage of the Cerebrospinal fluid (CSF) by a floating cysticercus.[31] They also stated that, in some cases, the symptoms are insidious whiles others occur suddenly. Chronic headaches and seizures are the most common symptoms. Other symptoms include changes in mental status, behavioral abnormalities, progressive dementia, and focal neurologic signs.[31] Therefore, carriage of the eggs of *Taenia*s spp. by cockroaches in the hospital environment is a major health concern. Although *Hymenolepis nana* infection is most often asymptomatic, heavy infections can cause weakness, anorexia, irritability, abdominal pain, and diarrhea. It is noteworthy that prolonged or concurrent infection, especially in children can cause more severe symptoms, even though it is not as severe in adults.[32] Generally, the parasites appear to have limited occurrence among the cockroaches, and may not be as important as rotavirus in vector transmission of gastrointestinal agents by cockroaches. The common occurrence of intestinal or enteric microbes including parasites, bacteria and virus (rotavirus) on the cockroaches reflect their role in environmental health with regard to fecal contamination.[33]

It can be concluded from this study that cockroaches in the hospital environment have a high

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tendency to carry nosocomial agents including highly resistant organisms. Cockroaches also constitute an important reservoir of intestinal pathogens, notably rotavirus. Currently, the extent of cockroach infestation in hospitals throughout Ghana, as well as management of the pest, is not known. This study can serve as a basis for creating awareness about the public health risks of cockroaches in the hospital environment in the country. We recommend that hospitals in Ghana be kept free of cockroaches by using insecticides, as well as also by clearing areas that can serve as a comfortable habitat for cockroaches and other insects.

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Author Contributions
Conceived and designed the experiments: ESD, PBT-Q, PFA-K. Analyzed the data: EA, ESD, PBT-Q, SKA. Wrote the first draft of the manuscript: ESD, EA, PBT-Q. Contributed to the writing of the manuscript: SKA, PFA-K, KOD, IB, MO-T, IA-B. Agree with manuscript results and conclusions: SKA, PFA-K, ESD, PBT-Q, IB, MO-T, IA-B, EA, KOD. Jointly developed the structure and arguments for the paper: ESD, IB, MO-T, PBT-Q, EA, SKA, PFA-K. Made critical revisions and approved final version: PFA-K, ESD, PBT-Q. All authors reviewed and approved of the final manuscript.

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